

IN THE SPECIFICATION

Please amend the specification as follows.

On page 7, lines 14-16, please amend the paragraph as follows.

Figure 3 showsFigures 3A-B show an ELISA and an immunoblot employing rD1 as antigen that demonstrates production of anti-CARDS antibodies in sequential serum samples of two patients infected with *Mycoplasma pneumoniae*.

On page 34, line 17 through page 35, line 4, please amend the paragraph as follows.

Furthermore, any of the compositions of this invention can comprise a pharmaceutically acceptable carrier and a suitable adjuvant. As used herein, "suitable adjuvant" describes an adjuvant capable of being combined with the polypeptide and/or fragment and/or nucleic acid of this invention to further enhance an immune response without deleterious effect on the subject or the cell of the subject. A suitable adjuvant can be, but is not limited to, MONTANIDE ISA51 (Seppic, Inc., Fairfield, NJ), SYNTEX adjuvant formulation 1 (SAF-1), composed of 5 percent (wt/vol) squalene (DASF, Parsippany, N.J.), 2.5 percent Pluronic, L121 polymer (Aldrich Chemical, Milwaukee), and 0.2 percent polysorbate (TweenTWEEN 80, Sigma) in phosphate-buffered saline. Other suitable adjuvants are well known in the art and include QS-21, Freund's adjuvant (complete and incomplete), alum, aluminum phosphate, aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE) and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trealose dimycolate and cell wall skeleton (MPL+TDM+CWS) in 2% squalene/TweenTWEEN 80 emulsion.

On page 48, lines 2-20, please amend the paragraph as follows.

Wild-type *Mycoplasma pneumoniae* M129/B9 and clinical isolates were grown in SP-4 medium as above. Mycoplasma monolayers in logarithmic growth phase were washed two times with 10 ml PBS (pH 7.4) and one time with Dulbecco Modified Eagle Medium (DMEM) without L-cysteine and L-methionine and resuspended in 10 ml Dulbecco Modified Eagle Medium (DMEM) without L-cysteine and L-methionine supplemented with 10 % heat-inactivated fetal bovine serum and 100 μ Ci L-[³⁵S]methionine. After 4 h incubation at 37°C, supernatants were removed and monolayers washed twice with 25 ml PBS. Mycoplasma cells were scraped into a volume of 10 ml sterile PBS, collected by centrifugation at 9,500 $\times g$ and washed multiple times in PBS. Cell pellets were resuspended in 1 ml complete lysis buffer (CLB) prepared shortly before use (150 mM NaCl, 10 mM Tris, 20 μ M EGTA, 0.5 M ~~Triton X-114~~ TRITON X 114, 1 mM CaCl₂ and protease inhibitors 1 μ M pepstatin A, 200 μ M PMSF, 1 mM N- α -p-tosyl-L-lysine chloromethyl ketone (TLCK), and 10 μ M leupeptin. Cell pellets in CLB were sheared through 25 gauge needles using 3 ml syringes to obtain clear lysis. 20 μ l aliquots of resuspended cell lysate were transferred to separate microfuge tubes for SDS-PAGE analysis and scintillation counter assessment (Beckman Instruments Inc. Irvine, CA). Radiolabeled lysates were diluted to 6 ml in CLB and passed through control and experimental SP-A columns (see below) in parallel.

On page 48, line 22 through page 49, line 5, please amend the paragraph as follows.

A 20 x 1.2 cm control glass column was packed with 3 ml uncoupled SephadexSEPHAROSE, another identical (experimental) column was packed with 3 ml SephadexSEPHAROSE coupled to SP-A. Coupling of SP-A to SephadexSEPHAROSE CL-4B was performed as follows: A total of 1.5 mg of SP-A was coupled to 2g of CNBr-activated SephadexSEPHAROSE CL-4B according to the manufacturer's instructions except the coupling buffer was 10 mM sodium bicarbonate, pH 8.3. SP-A coupled SephadexSEPHAROSE was

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stored in 5 ml of 5 mM Tris pH 7.5, containing 1mM NaN₃. Columns were equilibrated with 50 ml CLB prior to addition of radiolabeled cell lysates. Radiolabeled cell lysates were collected and reapplied to each column 3-4 times. After samples were added, columns were washed with 10 times volume of packed material to remove unbound proteins. *M. pneumoniae* SP-A-binding proteins were eluted using a NaCl gradient (0.2 to 3 M NaCl) containing 10 mM EDTA. Eluates were collected as 1 ml fractions, and 20 µl from each fraction was assayed for specific activity with a scintillation counter.

On page 50, line 26 through page 51, line 6, please amend the paragraph as follows.

Mycoplasma total proteins or purified recombinant CARDs protein were resolved on 4-12% SDS-polyacrylamide gels (NuPAGE, InVitrogen) (His-tag released, i.e., minus His tag) and transferred electrophoretically to nitrocellulose membranes (Towbin *et al.*, 1979). Membranes were blocked for two hours with 5% (wt/vol) blotto [nonfat dry milk in TBS containing 0.1% Tween-20~~TWEEN-20~~ (TBST)], followed by three washes with TBST, and incubated with *M. pneumoniae* infected patient sera (1:50 to 1:100 in 2% blotto) at RT for 2 h. Then, individual membranes were washed three times (15 min per wash) in TBST and incubated for 2 h (ambient temperature) with alkaline phosphatase-conjugated goat anti-human IgG Abs at a dilution of 1:2000 in TBST, which were washed 5 additional times with TBST, then color developed with BCIP/NBT tablets (Sigma).

On page 53, lines 3-13, please amend the paragraph as follows.

Bound IgG was detected with alkaline phosphatase (AP)-conjugated goat-antihuman IgG diluted 1:3000. Individual strips were developed for 1-5 minutes with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate p-toluidium (BCIP) solution. Results of the immunoblotting showed a colored band of 68 kDa molecular weight on each membrane containing rCARDs toxin and colored bands of 32 kDa and 28 kDa on each membrane

containing the D1 domain, thus demonstrating seroconversion in these patients and detection of antibodies to the CARDs toxin, either as a recombinant protein or as the D1 domain. In the latter assay, the color intensity of each band appears to increase in the samples in a manner consistent with the time course of collection from the patient during the course of the disease (i.e., 1-1<1-2<1-3) (Figure 3Figures 3A-B).

On page 53, lines 16-28, please amend the paragraph as follows.

ELISAs were also carried out on the samples collected from patients 1 and 2 described above (i.e., samples 1-1, 1-2, 1-3, 2-1, 2-2, and 2-3). In these assays, washing at each stage was performed at least three times with PBS and sera and antibodies were diluted in 1% BSA in PBS. Each well of ImmulenIMMULON 4 HBX Immunoplates (Dynox) was coated overnight at 4°C with 50 µl of rCARDs toxin/D1 (1 µg/well) diluted in carbonate/bicarbonate buffer (32 mM Na₂CO₃, 64 mM NaHCO₃). Individual plates were washed, 100 µl of 1 mg/ml (wt/vol) BSA in PBS was added to each well, and incubation continued for two hours at room temperature. After washing, 50 µl of diluted human serum samples (1/50 to 1/3200) were added to each well, and plates were incubated for two hours at room temperature. Then, plates were washed, and 50 µl of diluted (1:1000) alkaline phosphatase (AP)-conjugated goat-antihuman IgG (Zymed) were added to each well. Plates were incubated for 1.5 hours at room temperature, washed and 50 µl of substrate solution [p-nitrophenyl phosphate (PNPP)/0.1M Tris pH 9.6] was added and plates were incubated at room temperature for 30-60 minutes. Absorbance values at 450 nm were determined for each well.

On page 54, lines 14-22, please amend the paragraph as follows.

Additional studies were conducted wherein each well of an ImmulenIMMULON 4 HBX Immunoplate (Dynox) was coated overnight at 4°C with 50 µl of rCARDs toxin (1, 2 or 3 µg/well) diluted in carbonate/bicarbonate buffer. After washing, 50 µl of diluted human serum

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samples (1/200 dilution of convalescent serum 1-3 as described above) was added to each well and plates were incubated for two hours at room temperature prior to detection of bound IgG. Negative patient serum control was also included. The results showed an optical density around 1.8 and $1.9 \pm SE$ for all three concentrations of rCARDs toxin and an optical density of the negative control around 0.6 and $0.7 \pm SE$ for all concentrations of toxin.